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Bypassing Translation Initiation

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A high-resolution cryo-EM reconstruction of a ribosome-bound dicistrovirus IRES (Schüler et al., 2006) and the crystal structure of its ribosome binding domain (Pfingsten et al., 2006) provide new insights into an exceptional eukaryotic translation mechanism.

Internal ribosomal entry sites (IRESs) are elements present in a subset of eukaryotic mRNAs that mediate translation initiation by noncanonical, end-independent mechanisms known collectively as internal ribosomal entry. Over the last decade, the outlines of three such mechanisms have been elucidated that have different requirements for eukaryotic initiation factors (eIFs). The simplest mechanism is used by the ~200 nt long intergenic region (IGR) IRESs that separate the two large coding regions in the RNA genomes of dicistroviruses such as Cricket paralysis virus (CrPV) and *Plautia stali* intestine virus (PSIV). Initiation on IGR IRESs occurs at GCU/GCA/GCC (alanine) or CAA (glutamine) codons rather than at AUG initiation codons and involves neither initiator tRNA (Met-tRNA$^{Met}_i$) nor eIFs (Sasaki and Nakashima, 2000; Wilson et al., 2000; Pestova and Hellen, 2003; Jan et al., 2003). The recent complementary structural advances reported by Schüler et al. (2006) and Pfingsten et al. (2006) provide new insights into how IGR IRESs promote a process that usually requires at least 11 eIFs.

The canonical initiation mechanism ("scanning initiation") comprises a coordinated series of events that include binding of the eIF2•GTP•Met-tRNA$^{Met}_i$ ternary complex to the 40S ribosomal subunit, attachment of the resulting 43S complex to the 5′ end of an mRNA, scanning to the initiation codon to form a 48S complex, and joining with a 60S ribosomal subunit to form an 80S ribosome in which the Met-tRNA$^{Met}_i$ anticodon is base paired to the AUG codon in the ribosomal peptidyl (P) site.

IGR IRESs enable ribosomes to bypass this process and begin elongation directly. They bind to 40S subunits and to 80S ribosomes independently of eIFs such that the IRES's 3′-terminal CCU triplet occupies the P site (Wilson et al., 2000). This interaction accounts for the competition between IGR IRESs and Met-tRNA$^{Met}_i$ for the P site (Wilson et al., 2000; Pestova et al., 2004). Translation begins following delivery of cognate aminoacyl-tRNA to the ribosomal aminoacyl (A) site by eukaryotic elongation factor (eEF) 1 and its translocation by eEF2 to the P site, which exceptionally occurs without prior peptide bond formation or a deacylated tRNA in the P site (Wilson et al., 2000; Jan et al., 2003; Pestova and Hellen, 2003). In addition to binding to the 40S subunit and mimicking the initiation codon/Met-tRNA$^{Met}_i$ anticodon in the P site, IGR IRESs establish the correct reading frame for translation and might facilitate their own translocation out of the P site. Pfingsten et al. (2006) and Schüler et al. (2006) have established a structural framework for understanding these different steps.

IGR IRESs have closely related structures (Kanamori and Nakashima, 2001): the three domains each contain an essential pseudoknot (Figure 1A). The base-paired CCU triplet that occupies the P site is in domains 3′s pseudoknot (PK I). Domain 3 is connected to PKII (part of domain 1), and domain 1 folds with domain 2, the ribosome binding element (which contains PKIII), to form a stable double-nested pseudoknot. In their 3.1 Å crystal structure (Figure 2), Pfingsten et al. (2006) report that the constituent elements of PSIV IRES, domains 1 and 2 pack together tightly as a result of multiple stabilizing A-minor interactions involving both strands of the large L1.2 loop and the minor groove of helix P2.2. Mutagenesis and footprinting experiments established that the conserved SL-IV and SL-V stem-loops make direct, functionally important interactions with the 40S subunit (Nishiyama et al., 2003). They emerge from the same side of this highly structured core, almost at right angles to

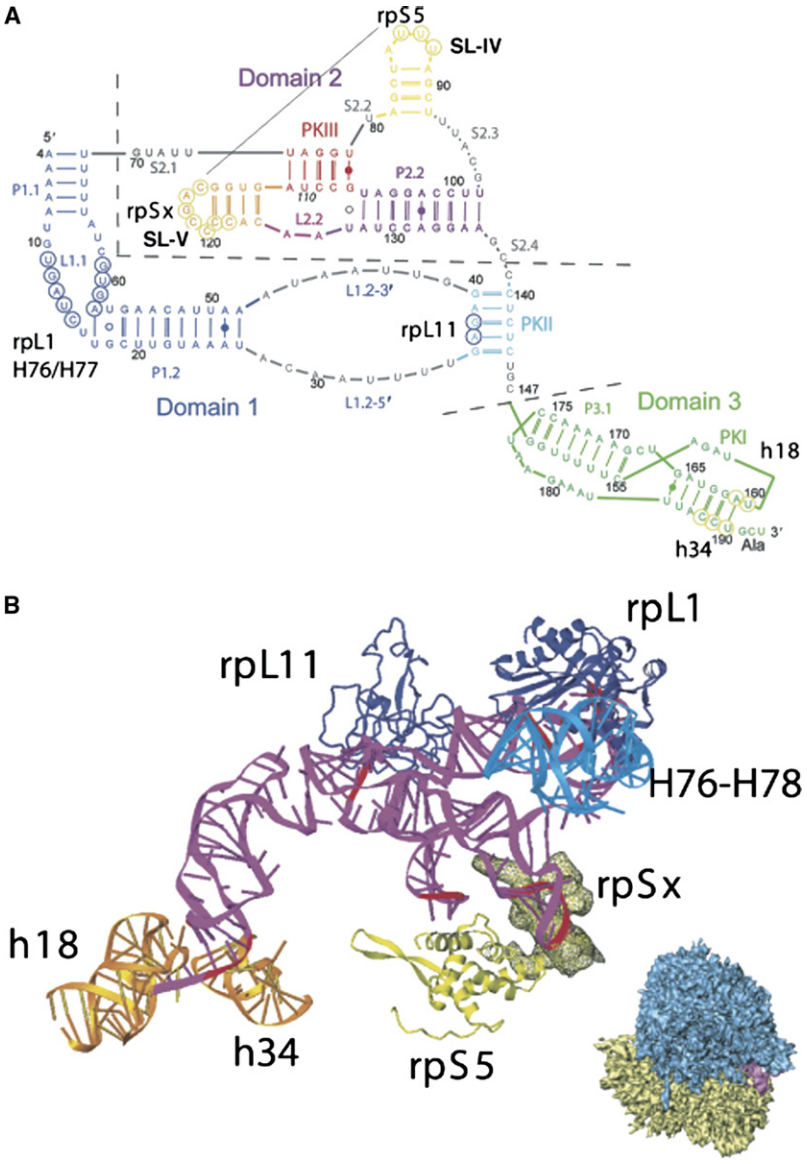

Figure 1. Interactions of the CrPV IGR IRES with the 80S Ribosome

(A) Secondary structure of the CrPV IRES, labeled to show domains, pseudoknots (PK), stem-loops IV and V (SL-IV and SL-V), paired helices (P), loops (L), and single-stranded elements (S). Circled nucleotides are candidates for interaction with the indicated ribosomal components.

(B) A view of the cryo-EM model of the IRES (magenta ribbon, with nucleotides that likely interact with ribosomal components, colored red) with components of the 80S ribosome that appear to interact with the IRES, including rpS5 (orange ribbon), h18/h34 (light green ribbon), rpSx (a ribosomal protein of unknown identity in green mesh), rpL1, rpL11, and H76-H78 (blue ribbon). The inset image of the IRES-80S ribosome complex is included to aid orientation. The figure was generously provided by Christian Spahn and is reproduced from Schüler et al. (Nat. Struct. Mol. Biol. *13*, 1092-1096 [2006]) with permission of the Nature Publishing Group.

the underwound helix P2.2. Domains 1 and 2 are therefore preformed as a structural unit that enables SL-IV and SL-V to interact with adjoining regions on the 40S subunit. Domain 1 forms part of the supporting substructure for the P2.2 platform from which SL-IV and SL-V emerge, but does not contact the 40S subunit. Instead, its orientation allows it to interact with the 60S subunit.

Spahn and colleagues previously reported cryo-EM reconstructions of 40S subunits and 80S ribosomes bound to the CrPV IRES at 20.3 Å and 17.3 Å resolution, respectively (Spahn et al., 2004). The 7.3 Å resolution reconstruction of the IRES in complex with yeast 80S ribosomes (Figure 1B; Schüler et al., 2006) therefore represents a significant technical advance, and allows modeling of the entire IRES. Each IRES domain interacts with distinct ribosomal elements in the intersubunit space. Domain 2 binds the 40S subunit through interactions of SL-IV and SL-V with ribosomal protein (rp)S5 and rpSx in the exit (E) site region. The IRES induces conformational changes in the 40S subunit like those caused by the hepatitis C virus IRES (Spahn et al., 2001), even though that IRES is unrelated to IGR IRESs and (apart from its interaction with rpS5) binds to a different location on the 40S subunit. These changes may stabilize interactions in the mRNA binding channel of the 40S subunit, and binding of these IRESs therefore mimics a step that may also be part of initiation complex assembly during the scanning initiation process. This conformational change in the 40S subunit is reversed upon joining of the 60S subunit, and whereas interactions of Domain 2 with the 40S subunit persist, Domain 3 retracts from the A site so that this site becomes accessible to aminoacyl-tRNA and the first (alanine) codon of the coding region (Figure 1A) can engage in typical A-site interactions with ribosomal RNA helices h18 and h34. Domain 1 interacts exclusively with the 60S subunit at sites that normally interact with tRNAs: loop L1.1 interacts with rpL1 and ribosomal RNA helices H76-H77 of the E-site, and PKII interacts with rpL11 in the P site. Pfingsten et al. (2006) report that L1.1 is required for joining of a 60S subunit to the IRES-40S subunit complex; however, reports that IGR IRESs can recruit 80S ribosomes directly suggest that the IRES's interaction with the 60S subunit E site might play additional roles, such as promoting the first eEF2-mediated translocation step (Nishiyama et al., 2003; Pestova and Hellen, 2003). In summary, although some details concerning initiation on IGR IRESs remain unresolved, progress in elucidating this mechanism

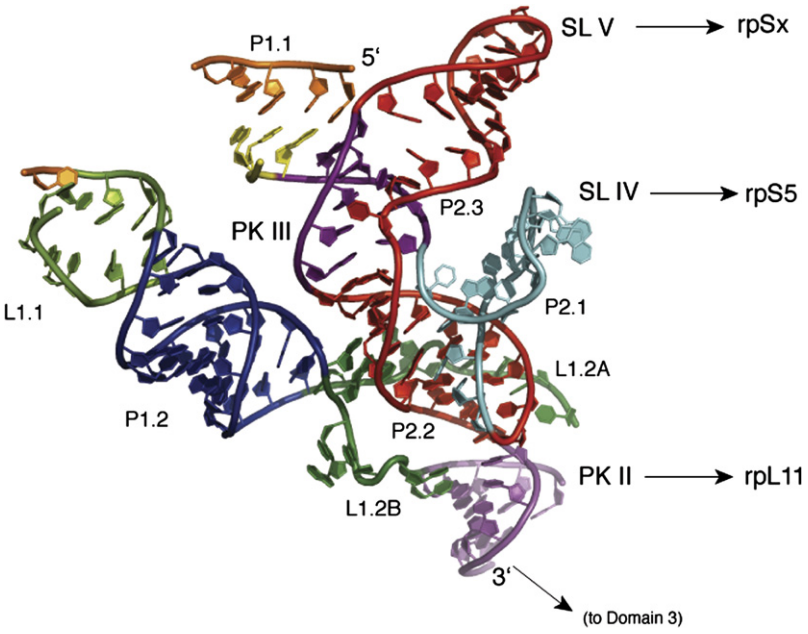

Figure 2. Structure of the Ribosome Binding Domains 1 and 2 of the PSIV IGR IRES
The structure is labeled to show pseudoknots (PK), stem-loops (SL), and paired helices (P), indicating likely interactions with components of the 80S ribosome. Figure generously provided by Jennifer Pfingsten and Jeffrey Kieft (Pfingsten et al., 2006).

has been rapid. The X-ray crystallography and cryo-EM studies of Pfingsten et al. (2006) and Schüler et al. (2006) substantially advance understanding of the activity of these remarkable RNAs.

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